IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

4172

In re Application of)
)
SHMUEL CABILLY ET AL.)
) Art Unit: 127
Serial No. 06/483,457)
) Examiner: J. HULEATT
Filed: April 8, 1983)
)
For: RECOMBINANT IMMUNOGLOBIN)
PREPARATIONS)

AFFIDAVIT UNDER C.F.R. § 1.131

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, RONALD B. WETZEL, being duly sworn, depose and say that I am a coinventor of the subject matter claimed in the above-described patent application. Affiant further deposes and says that the subject matter of at least claims 53-56, 58-60 and 63-67 was reduced to practice in the United States of America prior to March 25, 1983, as shown in the attached Exhibits. The specific dates appearing in the Exhibits have been obscured.

Exhibit 1 is a Western blot showing the levels of stable expression of murine anti-CEA gamma and kappa immunoglobulins (and a mixture of gamma and kappa) in E. coli transformed with plasmids bearing genes encoding the gamma, kappa, and gamma and kappa immunoglobulin chains, respectively. The levels of each of the proteins are shown

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the table at the top of the Exhibit.

Exhibit 2 is a representative example of how the bacterially-produced immunoglobulin chains were refolded into an immunologically active form, i.e. a form in which they were able to bind to CEA. Column D is a control (E. coli transformed with interferon), while columns A, B and C, respectively, represent refolding experiments on an extract from E. coli transformed with two plasmids, each bearing one of the gamma or kappa chain DNAs (A), an extract from E. coli transformed with a plasmid bearing the kappa chain only (B), and a combined extract from E. coli transformed with a plasmid bearing the gamma chain only (designated "43C") and a plasmid bearing the kappa chain only (C). Column D is a control. As expected, the results with the cotransformant extracts and combined extracts were essentially the same, in both cases indicating anti-CEA activity on the part of the refolded immunoglobulins (576 and 454 versus the control level of 2), while the refolded kappa chain, not having the companion variable region from the heavy (gamma) chain, was considerably less active.

Exhibit 3 is a similar experiment, although it includes additional runs with variations in the refolding reagents and conditions. Again, columns A and G-J show that the refolded extract from kappa and gamma cotransformed E. coli immunologically binds to its specific antigen, CEA, as does the refolded combined extract from separately

transformed E. coli (column C). Consistent with the results in Exhibit 2, refolded kappa chain was less active. Refolded recombinant gamma chains (column E) also were less active than the combined chains.

Further deponent sayeth not.

Ronald B. Wetzel

Ronald B. Wetzel

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